RESEARCH ARTICLE

General Characteristics and Cytotoxic Effects of Nano-Poly (Butyl Cyanoacrylate) Containing Carboplatin on Ovarian Cancer Cells

Leila Kanaani[1], Meysam Ebrahimi Far[1]*, S Maryam Kazemi[2], Edris Choupani[3], Maral Mazloumi Tabrizi[4], Hasan Ebrahimi Shahmabadi[5], Azim Akbarzadeh Khiyavi[6]

Abstract

The initial response to treatment and subsequent development of resistance to carboplatin are very important challenges. Use of nano drug delivery is a new method to replace standard chemotherapy. In this research, both non-PEGylated and PEGylated nanoparticles (NPs) were prepared by mini-emulsion polymerization of poly (butyl cyanoacrylate) (PBCA) NPs. Characteristics such as size, polydispersity index (PDI), zeta potential, drug release, and stability were examined. In addition, infrared spectroscopy was used for description of the produced NPs. Then, cytotoxicity effects of both formulations were studied on the A2780CIS ovarian cancer cell line with incubation for 24, 48, and 72h. Examination of characteristics of loaded carboplatin on the PBCA NPs under suitable laboratory conditions showed a positive effect of PEG on their properties. Cytotoxicity studies demonstrated greater toxicity with both formulations of nano-drugs than the free drug. The results indicated that PBCA NPs can be considered as suitable candidates for nano-drugs in chemotherapy.

Keywords: Ovarian cancer- carboplatin- poly (butyl Cyanoacrylate) nanoparticle- nano-drug delivery

Asian Pac J Cancer Prev, **18 (1)**, 87-91

Introduction

The most common metastases observed in ovarian cancer include lymphatic and released peritoneal (Kim et al., 2013). Debulking surgery is the initial treatment of choice for advanced ovarian cancer and alternative treatment strategy using platinum-based chemotherapy (Zhang et al., 2013). Carboplatin is DNA-binding and it causes bifunctional damages and finally induces cytotoxicity by connecting to the DNA molecules (Knox et al., 1986). The main purpose of the targeted drug delivery is controlled drug delivery to the target tissue with optimal therapeutic dose and rate as well as no toxicity and increase of effect efficiency of drug. Colloidal drug delivery systems include noisome, liposomes, micro-emulsions and NPs. Used NPs as drug carriers have overcome technical limitations and stability problems relevant to liposomes, noisome, and micro-emulsions (Williams et al., 2003). Polymeric nanoparticles can be manufactured by polymerizing monomers or polymers. In the early 1970s, two scientists pioneer in the field, Birrenbach, and Speiser produced the first nanoparticles for pharmaceutical applications. Two types of polymerization processes are used for the production of polymeric nanoparticles: dispersion polymerization (DP) and emulsion polymerization (EP) (Gilbert., 1995). Emulsion polymerization process is faster and easier than the other method so that it can be easily implemented on the industrial level (Kreuter et al., 1990). According to the type of selected process, which is anionic polymerization, the polymerization of the monomer is done through C = C double bond (Figure 1). PBCA NPs are of common nano-carriers as targeted drug delivery. This NPs have suitable characteristics for targeted drug delivery in the tissue including the ability to alter bio-distribution of drug in the body, biodegradability, and ease of synthesis and purification (Andrieux et al., 2009). There are two common methods for preparing of PBCA NPs that consist anionic polymerization reaction and mini-emulsion polymerization (Wu et al., 2009).

*[1]Department of Toxicology, Faculty of Pharmacy, Islamic Azad University, Shahreza Branch, Shahreza, [2]Department of Genetics, [4]Department of Toxicology and Pharmacology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran Medical Branch, [6]Department of Pilot Nanobiotechnology, Pasteur Institute of Iran, Tehran, [3]Department of Agronomy and Plant Breeding, College of Agriculture, University of Zanjan, Zanjan, [5]Department of microbiology, Parasitology and Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran *For Correspondence: ebrahimifar67@gmail.com*

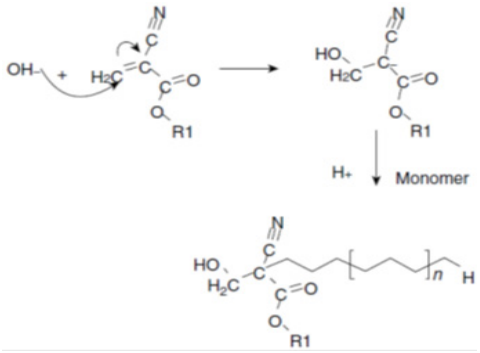

Figure 1. Anionic Polymerization of Alkyl Cyanoacrylate Monomers Leading to the Formation of the Nanoparticles. The Hydroxyl Ions in the Water Start the Reaction

In connection with the mini-emulsion polymerization, a type of emulsion are prepared that was commonly called for almost stability of oil-in-water nano-droplets that cut through the intense mixture of monomers, water, a stabilizer, and a hydrophobic insoluble in water (Wu et al., 2009). The mini-emulsion polymerization of monomers directly to the particles becomes more droplets. This is because the droplets act as the starting and propagation of polymerization. Therefore, it spite of emulsion polymerization, no need to transfer monomers or other hydrophobic compounds from one tank into a polymerization place. This makes mini-emulsion polymerization as a one-step nano-encapsulation method for the encapsulation of hydrophobic compounds (Antonietti et al., 2002).

Today, different carries have been used for carboplatin delivery. However, human hasn't been able to produce suitable NPs formulation of carboplatin since now. In this research, carboplatin is loaded on PBCA NPs by mini-emulsion polymerization. The efficiency of NPs are studied using the A2780CIS cell line of ovarian cancer resistance to carboplatin.

Methods and Materials

Materials

At first, monomer of butyl cyanoacrylate was purchased from Evobond®Tong Shen Enterprise Co., Ltd., Taiwan. Carboplatin, dextran 7000 and polyethylene glycol 3350 were prepared from Sigma–Aldrich Co., UK. Hydrochloric acid and sodium hydroxide were obtained from Merck Company. The A2780CIS cell line was prepared from cell bank of Iran Pasteur Institute.

Preparation method of NPs containing drug

First, 300 µl monomers of butyl cyanoacrylate was added and mixed to the compound containing 220 µl HCl 0.01N, 150 mg honey (Sabalan Co. Iran), 40 µl olive oil (Farzan Rahbar Saba Co. Iran), and 45 mg dextran 70,000. Then, 90 mg PEG3350 (in both no-PEGylated and PEGylated formulations) and 50 mg carboplatin were added to the resultant formulation and mixed under lab conditions (150 rpm on stirrer). Next, 25 ml cold distilled water was added to the mixture during two steps. The mixture was stirred on stirrer (400 rpm, 10 min) to obtain pre-emulsion. Therefore, sonication was performed by probe sonicator (50. W, Bandel in Sonopuls HD 2070, Bandelin Elec., Germany) with placing flask on the cold water. Emulsion was again put on stirrer after 24h maintenance in refrigerator (4°C), was slowly stirred (150 rpm, 3.5h), and the polymerization process was completed. Afterwards, pH of mixture was neutralized using NaOH 0.1N.

Characterization of NPs

The Size, PDI, and zeta potential were studied by zeta-sizer (Nano ZS3600, Malvern Instruments, UK) machine. In order to measurement, nano drugs of both formulations and PBS (pH: 7.2, 10M) with 1:7 ratio was prepared. Drug loading and encapsulation efficiency are also determined spectrophotometrically. Briefly, results of both formulations were centrifuged (15 min at 4 °C and 49000g) by ultracentrifuge instrument to define encapsulation and drug loading non-connected drugs. Finally the platinum contained in the formula 1&2. moreover, linkage analysis of PEGylated PBCA NPs were obtained by infrared spectroscopy (Thermo Nicole, Nexus 870, USA).

$$\text{encapsulation percent} = \frac{\text{prime carboplatin (mg/ml)} - \text{available carboplatin in the supernatant (mg/ml)}}{\text{prime carboplatin (mg/ml)}} \times 100$$

(Formula 1)

$$\text{loading percent} = \frac{\text{the amount of available drug in the nanoparticle (mg/ml)}}{\text{weight of nanoparticle (mg/ml)}} \times 100$$

(Formula 2)

Both formulations were maintained in the room temperature for 2 months. Physicochemical characteristics (such as size, PDI, zeta potential, encapsulation, and drug loading efficiency) of PBCA NPs were studied.

Examining drug release

To measure drug release, human serum with 0.8 mg/ml NPs (both formulations) containing drug is prepared. Human serum containing NPs are put in shaker of incubator (37 °C, 30 min, 130 rpm). Based on the decomposition rate, drug release was studied in human serum containing drug NPs. Light absorption of serum containing NPs at 220 nm is studied during 38h in different intervals.

Examining cytotoxicity

Cytotoxicity test of NPs were evaluated in A2780CIS cell line by MTT assay on A2780CIS cell line. Used concentrations (0, 4, 8, 16, 32, 64, and 96 µM) of nano drug, free drug, and control drug (both formulations) were evaluated during 24, 48, and 72h incubation.

Statistical analysis

The resulted data was studied in SPSS soft (v.15). Statistically, p-values less than 0.05 were considered important. All results were expressed as mean ±SD deviation (SD, n = 3).

Results

Table 1. Examining the Characteristics of Loaded Carboplatin on the PBCA NPs

Samples	Size (nm)	Zeta potential (mV)	PDI	D.L.E (%)	E.E (%)
No-PEG Nano drug	490.5±42.5	-11.8±0.84	0.263±0.019	3.1±0.3	36±2.9
PEG Nano drug	360.2±30.1	-10.3±0.69	0.384±0.33	3.6±0.3	40±3.3

PDI, polydispersity index; E.E, Entrapment efficiency; D.L.E, Drug loading efficiency

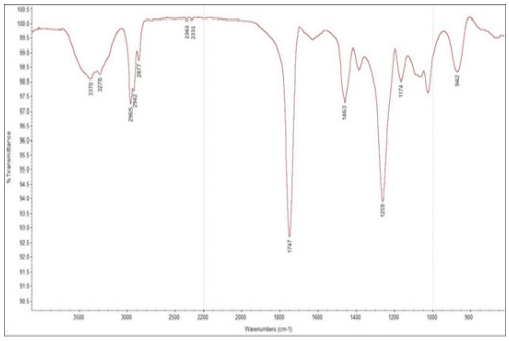

Figure 2. Infrared Spectra of PEGylated PBCA NPs

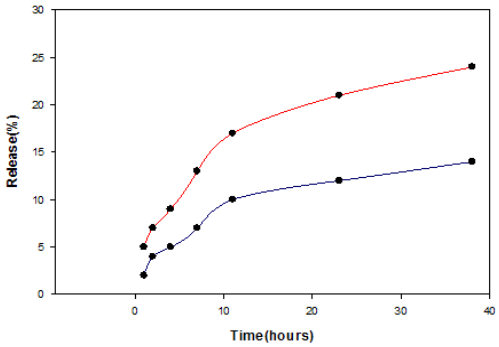

Figure 3. Pattern of Carboplatin Release of PBCA NPs (Blue and Red Lines are Relevant to the PEGylated and no-PEGylated Nano Drugs, Respectively)

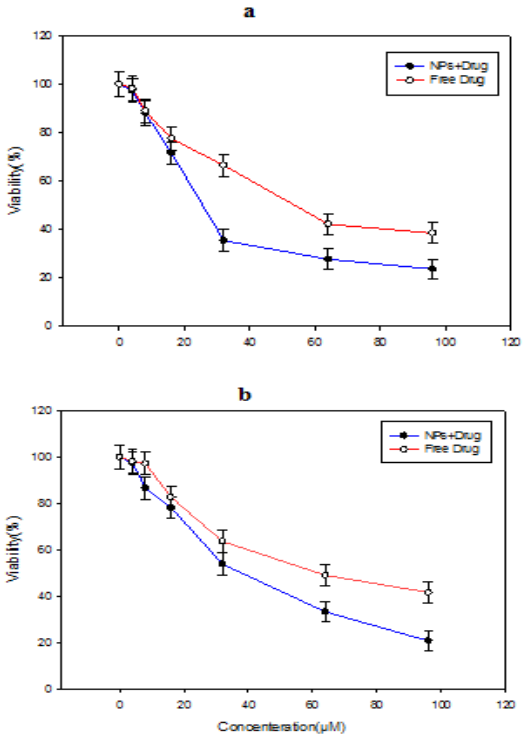

Figure 4. Cytotoxicity Effects of Free Carboplatin and Loaded Carboplatin on the PBCA NPs in the A2780CIS Cell after 24h Incubation. (a) PEGylated NPs (P<0.05) and (b) no- PEGylated NPs (P<0.05)

Characterization of NPs

Polymerization occurred after gradual adding of cold water and was completed after sonication, hence, environment color started to change to milky. The obtained results from physicochemical characteristics of the carboplatin NPs are summarized in Table 1. In addition, Infrared linkage analysis of PEGylated PBCA NPs showed indicator bands of fundamental agent groups of the compound including characteristic bonds of ester group (C=O and C-O) and nitrile group (C≡N). The frequency band at 1747, 1259, and near to the band at 2,200 cm^{-1} are due to C=O, C-O, and C≡N stretching, respectively. Moreover, it is not characteristic band of C=C available in monomer. Instead, a broad band in the range 3,275-3,370 cm^{-1} is attributed to the O-H (Figure 2). Obtained results of nano drug (both formulations) showed if NPs are maintained in room temperature at 25°C for two months, they are stable in lab conditions. There are insignificant changes during maintenance than before it (Table 2 and 3).

Drug release

The results of drug release indicated that nano drugs have a suitable retention power. Regarding the nano drug, an initial burst of drug release during the second-1 hours (4.4% of encapsulated drug of PEGylated nano drug) followed by mild ascending slope with maximal release of 3.8% after 38 h. In contrast, no-PEGylated nano drug is shown faster release pattern in which 96±2.8% of No-PEGylated nano drug is found into human serum. (Figure 2)

Table 2. Physicochemical Characteristics of PBCA NPs (No-Pegylated Formulation) in the Production Time and Two Months Later

No-pegylated formulation	Size (nm)	Zeta potential (mV)	PDI	D.L.E (%)	E.E (%)
S1	490.5±42.5	-11.8±0.84	0.263±0.019	3.1±0.3	36±2.9
S2	492.0±30.0	-11.3±0.77	0.289±0.022	3.3±0.2	37±3.1
S3	498.0±31.9	-10.3±0.76	0.308±0.029	3.4±0.3	39±3.3
S4	509.0±35.6	-9.8±0.71	0.395±0.031	3.6±0.3	42±3.6

PDI, polydispersity index; E.E, Entrapment efficiency; D.L.E, Drug loading efficiency; S, In the period of 14 days

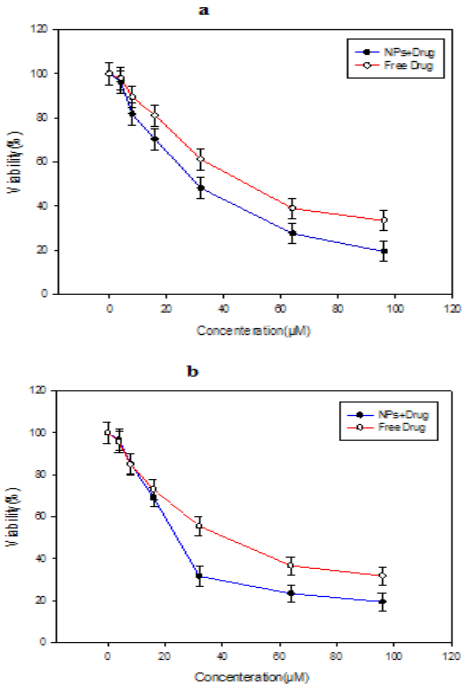

Figure 5. Cytotoxicity Effects of Free Carboplatin and Loaded Carboplatin on the PBCA NPs in the A2780CIS Cell after 48h Incubation. (a) PEGylated NPs (P<0.05) and (b) no-PEGylated NPs (P<0.05)

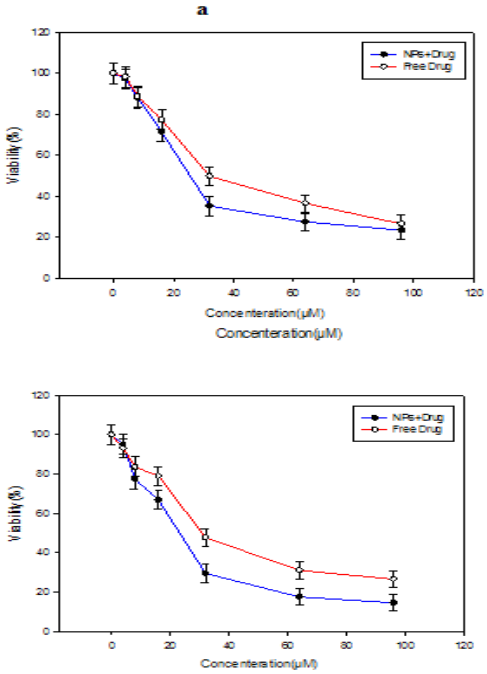

Figure 6. Cytotoxicity Effects of Free Carboplatin and Loaded Carboplatin on the PBCA NPs in the A2780CIS Cell after 72h Incubation. (a) PEGylated NPs (P<0.05) and (b) no-PEGylated NPs (P<0.05)

Table 3. Physicochemical Characteristics of PBCA NPs (Pegylated Formulation) in the Production Time and Two Months Later

pegylated formulation	Size (nm)	Zeta potential (mV)	PDI	D.L.E (%)	E.E (%)
S1	360.0±30.1	-12.3±0.7	0.384±0.033	3.6±0.3	40.0±3.3
S2	363.0±33.8	-11.7±0.7	0.398±0.035	3.9±0.3	41.0±3.5
S3	368.0±35.1	-10.9±0.8	0.420±0.041	4.0±0.4	44.0±3.8
S4	373.0±34.7	-10.3±0.8	0.460±0.043	4.1±0.4	46.0±4.1

PDI, polydispersity index; E.E, Entrapment efficiency; D.L.E, Drug loading efficiency; S, In the period of 14 days

Cytotoxicity of nano drug

At first, there are specified that 48±3.2 µg/ml and 57±4.4 µg/ml (of PEGylated Nano drug and no-PEGylated Nano drug, respectively) concentration of control NPs have no cytotoxicity and that is completely safe. The results showed cytotoxicity of nano drug and free drug considerably change during 24h (Figure 3), 48h (Figure 4), and 72h (Figure 5), so that IC50 values of Nano drug and free drug are estimated 25.7±1.8 and 54.0±3.7 µM in PEGylated nano drug and 38.2±2.0 and 61.3±2.9 µM in no-PEGylated Nano drug during 24h incubation, respectively. They are estimated 24.1±1.1 and 41.9±1.9 µM in PEGylated nano drug and 30.7±1.4 and 47.4±2.1 µM in no-PEGylated Nano drug during 48h incubation, respectively. Also, they are computed 23.0±1.1 and 30.0±1.3 µM in PEGylated nano drug and 25.6±1.2 and 33.7±1.5 µM in no-PEGylated Nano drug during 72h incubation, respectively. It is observed cytotoxicity of nano drug increased with increasing concentration than standard drug, too.

Discussion

Pharmaceutical biotechnology is the main challenge, and targeted drug delivery NPs have the ability to deliver drugs into target cells. Pharmaceutical nano-carriers can be made by PBCA NPs (Wu et al.,2009). Butyl cyanoacrylate monomer in the production of NPs of several factors involved, such as monomer concentration, concentration of stabilizer or dextran, pH environment, and quickly Stirring Pointed (Douglas et al., 1984; Douglas et al., 1985; Niall et al., 2001). In the manufacturing process, the two combined honey and olive oil was used as a surfactant for anti-cancer activity (Savrikar et al., 2010; Fontes et al., 2010). The used PEG in this study could increase the stability and augment drug delivery to tumor (Otsuka et al., 2012), water solubility, low immunogenicity, and antigenicity and is able to extend the period of drug release (Shahbazian et al., 2015). Therefore, the power of retention capability may be partially come from the presentment of PEG in structure of the NPs. In this study, carboplatin is loaded on the PBCA NPs as no-PEGylated and PEGylated formulations (Zarei et al., 2013). Zeta potential of -11.3 Mv and -10.3 Mv in both formulations confirmed the proper stability of particles. Meanwhile, Zeta potential ofNPs is correlated with suspension stability (Honary et al., 2013). After characterization of resulted NPs, it is revealed that in addition to preparation

method, PEG has positive effect on the properties of NPs. It is observed the efficiency of encapsulation and loading rate increased in PEGylated nano drug than no-PEGylated Nano drug (Zarei et al., 2013).

There is significant decrease of size in the presence of PEGylated Nano drug than no-PEGylated Nano drug. Apparently, this is due to the nature of hydrophilicity and high permeability of PEG that permeates to the NPs layers and tightens them. High encapsulation percent and loading rate of PEGylated nano drug confirms this matter than no-PEGylated nano drug, because the drug release possibility of the walls of tighten vesicles reduced. Therefore, retention yield and loaded drug rate of them increase (Zarei et al., 2013). In the present study, the stability of PBCA was confirmed 2 months after production, and this phenomenon is sensible in the drug release pattern of PBCA. Thus, in the same condition of preparation, drug release of PEGylated nano drug is lower than PEGylated nano drug. This is attributed to the coating and inhibitory effect of PEG on the drug release of NPs (Zarei et al., 2013) . Cytotoxicity effect of both formulations was studied by MTT test. The results showed non-toxicity of control NPs in high dose. The findings of loaded drug NPs indicated the lowest IC50 or the most toxicity than standard drug in both formulations. It is indicator of increase of drug efficiency and as a result using drug nano-carriers. On the whole, our research confirmed that PEGylated carboplatin PBCA has more cytotoxicity effects than no-PEGylated nano drug on the cells during 24, 48, and 72h incubation. In addition, increasing incubation time in both formulations increased the drug toxicity nano drug.

Mini-emulsion polymerization technique is approved as efficiency of preparation method of PBCA NPs containing carboplatin. Physicochemical characteristics of the carboplatin of NPs containing carboplatin were evaluated and found proper. The study is followed by evaluation the efficacy of nano drug on the ovarian cancer cell line A2780CIS which demonstrated superior cytotoxicity of nano drug in comparison to free drug in both formulations. The results of this study demonstrated that PEGylated and no-PEGylated PBCA NPs are proper carrier for carboplatin delivery to ovarian cancer cell line A2780CIS.

Acknowledgements

This work was supported financially by Pilot Nanobiotechnology Department, Pasteur Institute of Iran.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Andrieux K, Couvreur P (2009). Polyalkylcyanoacrylate nanoparticles for delivery of drugs across the blood–brain barrier. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, **1**, 463-74.

Antonietti M, Landfester K (2002). Polyreactions in miniemulsions. *Prog. Polym Sci*, **27**, 689-757.

Behan N, Birkinshaw C, Clarke N (2001). Poly n-butyl cyanoacrylate nanoparticles: a mechanistic study of polymerisation and particle formation. *Biomaterials*, **22**, 1335-44.

Douglas S, Illum L, Davis S (1985). Particle size and size distribution of poly (butyl 2-cyanoacrylate) nanoparticles. II. Influence of stabilizers. *J Colloid Interface Sci*, **103**, 154-63.

Douglas S, Illum L, Davis S, et al (1984). Particle size and size distribution of poly (butyl-2-cyanoacrylate) nanoparticles: I. Influence of physicochemical factors. *J Colloid Interface Sci*, **101**, 149-58.

Fontes GC, Amaral F, Filomena P, et al (2010). Factorial design to optimize biosurfactant production by Yarrowia lipolytica. *Bio Med Res Int*, **2010**, 1-8.

Gilbert RG (1995). Emulsion polymerization: a mechanistic approach, Academic Press, London.

Honary S, Zahir F (2013). Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 2). *Trop J Pharm Res*, **12**, 265-73.

Kim JH, Chung HH, Jeong MS, et al (2013). One-step detection of circulating tumor cells in ovarian cancer using enhanced fluorescent silica nanoparticles. *Int J Nanomedicine*, **8**, 22-47.

Knox RJ, Friedlos F, Lydall DA, et al (1986). Mechanism of cytotoxicity of anticancer platinum drugs: evidence that cis-diamminedichloroplatinum (II) and cis-diammine-(1, 1-cyclobutanedicarboxylato) platinum (II) differ only in the kinetics of their interaction with DNA. *Cancer Res*, **46**, 1972-9.

Kreuter J (1994). Colloidal drug delivery systems, Marcel Dekker, New York, pp 219-42.

Otsuka H, Nagasaki Y, Kataoka K (2003). PEGylated nanoparticles for biological and pharmaceutical applications. Adv. *Drug Deliv Rev*, **55**, 403-19.

Savrikar S, Lagad C (2010). Study of preparation and standardization of'Maadhutailika Basti'with special reference to emulsion stability. *AYU*, **31**, 1-6.

Shahbazian S, Akbarzadeh A, Torabi S, et al (2015). Anti-cancer activity of pegylated liposomal trans-anethole on breast cancer cell lines MCF-7 and T47D. *Biotechnol Lett*, **37**, 1355-9.

Williams J, Lansdown R, Sweitzer R, et al (2003). Nanoparticle drug delivery system for intravenous delivery of topoisomerase inhibitors. *J Control Release*, **91**, 167-72.

Wu M, Dellacherie E, Durand A, et al (2009a). Poly (n-butyl cyanoacrylate) nanoparticles via miniemulsion polymerization (1): Dextran-based surfactants. *Colloids Surf*, **69**, 141-6.

Wu M, Frochot C, Dellacherie E, et al (2009b). Well-defined poly (butyl cyanoacrylate) nanoparticles via miniemulsion polymerization. *Macromol Symp*, **281**, 39-46.

Zarei M, Norouzian D, Honarvar B, et al (2013). Paclitaxel loaded niosome nanoparticle formulation prepared via reverse phase evaporation method: an in vitro evaluation. *Pak J Biol Sci*, **16**, 295-8.

Zhang J, Kan Y, Tian Y, et al (2013). Effects of poly (ADP-ribosyl) polymerase (PARP) inhibitor on cisplatin resistance & proliferation of the ovarian cancer C13* cells. *Indian J Med Res*, **137**, 527-32.